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eEF1A2 and neuronal degeneration

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Abstract

Translation elongation factor eEF1A exists as two individually-encoded variants in mammals which are 98% similar and 92% identical at the amino acid level. One variant, eEF1A1, is almost ubiquitously expressed, the other variant, eEF1A2, shows a very restricted pattern of expression. A spontaneous mutation was described in 1972 which gives rise to the wasted phenotype: homozygous *wst/wst* mice develop normally until shortly after weaning, but then lose muscle bulk, acquire tremors and gait abnormalities and die by four weeks. This mutation has been shown to be a deletion of 15kb that removes the promoter and first exon of the gene encoding eEF1A2. The reciprocal pattern of expression of eEF1A1 and eEF1A2 in muscle fits well with the timing of onset of the phenotype of wasted mice: eEF1A1 declines after birth until it is undetectable by three weeks whereas eEF1A2 expression increases over this time. No other gene is present in the wasted deletion, and transgenic studies have shown that the phenotype is due to loss of eEF1A2. We have shown that eEF1A2, but not eEF1A1, is also expressed at high levels in motor neurons in the spinal cord. Wasted mice develop many pathological features of motor neuron degeneration and may represent a good model for early onset motor neuron disease. Molecular modelling of the eEF1A1 and eEF1A2 protein structures highlights differences between the two variants that may be critical for functional differences. Interactions between eEF1A2 and ZPR1 may be important in motor neuron biology.

### eEF1A variants and the wasted mouse mutation

Translation elongation is a tightly controlled, crucial process involving a number of elongation factors: eEF1A is responsible for the delivery of aminoacylated tRNAs to the A site of the ribosome in a GTP dependent process mediated by the multi subunit protein eEF1B; translocation is then facilitated by eEF2. During the last few years translation factors have been directly implicated in human disease; mutations in subunits of initiation factor eIF2B have been found to be mutated in vanishing white matter (1) and elongation factor eEF1A2 has been found to be a potential oncoprotein in ovarian cancer and has been shown to be overexpressed in a number of other tumour types (2-5).

eEF1A (formerly known as EF1 $\alpha$ ) is the second most abundant protein in the cell. It exists in mammals as two variant forms encoded by separate genes which give rise to proteins that are 92% identical and 98% similar but which have different expression patterns: eEF1A1 is almost ubiquitously expressed but eEF1A2 is expressed only in brain, spinal cord, heart and muscle at the level of detection in whole tissues.

We have shown that a 15 kb deletion abolishing expression of eEF1A2 is responsible for the mutant mouse phenotype wasted (6), a model for motor neuron degeneration (7). The wasted (*wst*) mutation of the mouse arose spontaneously in the Jackson Lab in 1972 (8). Mice which are homozygous for the mutation develop loss of muscle bulk, tremors and gait abnormalities from just after weaning (three weeks of age) and then die by four weeks; this timing is unaffected by genetic background or environmental changes. At a microscopic level, the only notable changes in the mice are seen in the spinal cord; the phenotype of homozygous mice closely resembles that of early onset motor neuron disease (MND, also known as amyotrophic lateral sclerosis or ALS). Progressive retraction of neurons from motor nerve endplates is seen, starting at 17 days at the thoracic level and extending to the lumbrical level by 23 days. The mice have weak synaptic transmission at 23 days at the thoracic level and by 25 days at the lumbrical level, so again there is a progressive deterioration with a rostrocaudal gradient. Wasted mice show signs of reactive gliosis and perikaryal neurofilament accumulation from 19 days, and conspicuous vacuolation of

motor neurons from 25 days. Muscle bulk is lost from 20 days, and *wst/wst* mice perform less well than their normal littermates on rotarod assays from 21 days (9). In all, the phenotype of the mice reflects many of the changes seen in human motor neuron disease, but with early onset, and compressed into a very short time-frame.

The wasted deletion removes the promoter and first exon of the gene encoding eEF1A2 (6). The reciprocal pattern of expression of eEF1A1 and eEF1A2 fits well with the timing of onset of the phenotype of wasted mice: eEF1A1 in muscle declines after birth until it is undetectable by three weeks whereas eEF1A2 expression increases over this time (6,10). No other gene is present in the wasted deletion, and our transgenic studies using BAC-mediated correction have shown that the phenotype is due to loss of eEF1A2. Mouse BACs, large genomic constructs spanning the gene encoding eEF1A2 with several genes on each side, were used to make transgenic mice. These were then crossed to *+/wst* mice and then backcrossed to further *+/wst* mice to generate *wst/wst* mice carrying the transgene; the phenotype of these mice was then compared with non-transgenic wasted mice. BACs spanning the deleted region corrected all aspects of the wasted phenotype, but a BAC with a specific deletion of exons 2 to 4 of eEF1A2 (i.e. the exons encoding the GTP binding sites) failed to correct any aspect of the phenotype (11), showing that the only gene implicated in the phenotype is that encoding eEF1A2. We have further shown that eEF1A2, but not eEF1A1, is expressed at very high levels in motor neurons in the spinal cord (11). This correlates well with the pathology seen in the spinal cords of wasted mice, particularly the loss of motor neurons (presumably because these cells are no longer capable of carrying out protein synthesis). Using a panel of isoform-specific antibodies, we have also identified hitherto unsuspected sites of expression of eEF1A2 in normal tissue, such as enteroendocrine cells at the base of crypts in the intestine, glucagon expressing cells in the pancreatic islets and ganglion cells in the retina (11). It is not yet known, however, whether these sites of expression are specific to eEF1A2, or whether eEF1A1 is also expressed in these cell types.

### Non-canonical functions of eEF1A

In addition to its role in translation, eEF1A1 has been shown to have numerous additional non-canonical (or “moonlighting”) functions, ranging from cytoskeletal

interactions (12) and apoptosis (13) to a role in proteasomal mediated degradation of damaged proteins (14). It has not yet been unequivocally established whether eEF1A2 shares any or all of these moonlighting functions, although in the case of apoptosis, it has been shown that in cultured differentiated myotubes, eEF1A1 is pro-apoptotic whereas eEF1A2 is anti-apoptotic (15). There are also many papers which demonstrate critical roles for eEF1A in diverse processes but where the precise variant is not identified because the only commercially available antibodies for eEF1A recognise eEF1A1 and eEF1A2 equally, and because peptides identified in the course of proteomics experiments are often from regions that are completely conserved between eEF1A1 and eEF1A2 (13). For example, a rapid increase in local eEF1A synthesis in response to long term potentiation (LTP) induction has been shown, suggesting a role for eEF1A in maintenance of LTP (16), and eEF1A has been demonstrated to have a role in mediating the heat shock response (17). A study of proteins that interact with a subunit of the inhibitory glycine receptor identified eEF1A as an interacting protein, together with other members of the translational machinery (18). eEF1A has also recently been shown to be a novel component of the nuclear export machinery in mammalian cells (19), and is involved in the nuclear export of proteins with a specific motif, including the von Hippel Lindau tumour suppressor. In none of these instances is it yet known whether the properties described are shared by both isoforms, or whether they are eEF1A1- or even eEF1A2-specific.

The observation that forced expression of eEF1A2 can transform cells and give rise to tumours in nude mice (3), when these cells already express eEF1A1 at high levels, suggests that there are real functional differences between the two variants. It may be that these relate entirely to their role in translation, but it is equally (and arguably more) likely that there are differences in non-canonical functions. The cell types that switch off eEF1A1 tend to be those that have a strong, stable cytoskeletal organisation, such as neurons and muscle; it is an attractive hypothesis that these cell types need to switch off eEF1A1 in order to prevent or modify the cytoskeletal rearranging properties of eEF1A1, but because of the obvious need to maintain protein synthesis they use eEF1A2. It is also noteworthy that muscle cells switch eEF1A1 back on in response to denervation or toxic injury, reverting back to high levels of eEF1A2 after recovery (20,21).

### Molecular modelling of eEF1A variants

eEF1A is a GTP-binding protein; the GTP exchange factor necessary for this activity is called eEF1B, which is a complex of three subunits, alpha, delta and gamma (22). eEF1A1 and eEF1A2 have similar properties in an *in vitro* translation assay, but in spite of the GTP binding sites being conserved, have different affinities for GTP and GDP. eEF1A1 has a seven-fold higher GDP dissociation rate than eEF1A2, and the GDP/GTP preference ratio is 0.82 for eEF1A1 but 1.5 for eEF1A2 (23); these data together suggest that eEF1A2 would have a greater dependence on a GTP exchange factor than eEF1A1. It was surprising, then, that yeast 2-hybrid experiments showed that whilst eEF1A1 binds all three eEF1B subunits, eEF1A2 showed little or no affinity for any of them (24), even though the eEF1A2 “bait” construct was able to interact with other proteins (25). This is even more surprising when the two variants are subjected to homology modelling (26), and the positions of the non-conserved amino acid residues mapped on their surfaces, showing that virtually all the amino acid differences between eEF1A1 and eEF1A2 are clustered in two areas of the protein, both of which are on the opposite side of the molecules from the eEF1B $\alpha$ -binding site (figure 1). This puzzle has yet to be resolved, but it remains a possibility that whilst eEF1B is the GTP exchange factor for eEF1A1, eEF1A2 uses a different, as yet unidentified, GTP exchange factor. Additionally, the close congregation of variable amino acids that are highly conserved among their respective eEF1A1 and eEF1A2 orthologues could comprise important binding sites that confer altered/different functional properties for the two human variants.

### eEF1A, ZPR1 and SMN

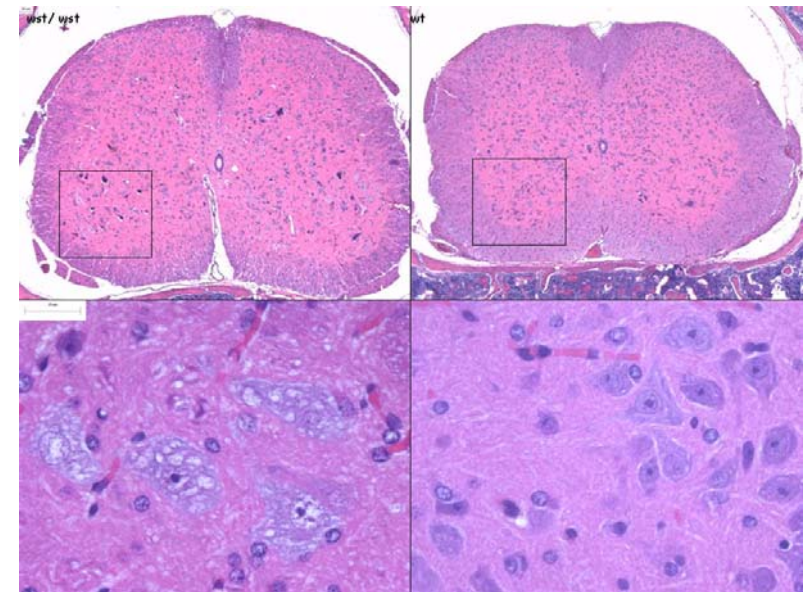
It has been known for some years that eEF1A (again, it is unknown whether this is eEF1A1, eEF1A2 or both) can be found in a complex with a zinc finger-containing transcription factor, ZPR1 (27). ZPR1 is in turn complexed with the survival motor neuron protein, SMN; this interaction is essential for the correct localisation of SMN in neurons. SMN is mutated in spinal muscular atrophy, an early onset form of motor neuron disease (28). It has not been established whether there is any direct contact between eEF1A and SMN, but a protein of the same size as eEF1A copurifies with

the ZPR1/SMN complex that seems likely to be eEF1A (29). Interestingly, mice that are heterozygous for a *Zpr1* knockout mutation develop motor neuron disease, with motor neurons being progressively lost over a 12 month period (30). The mice show mislocalisation of SMN in the motor neurons.

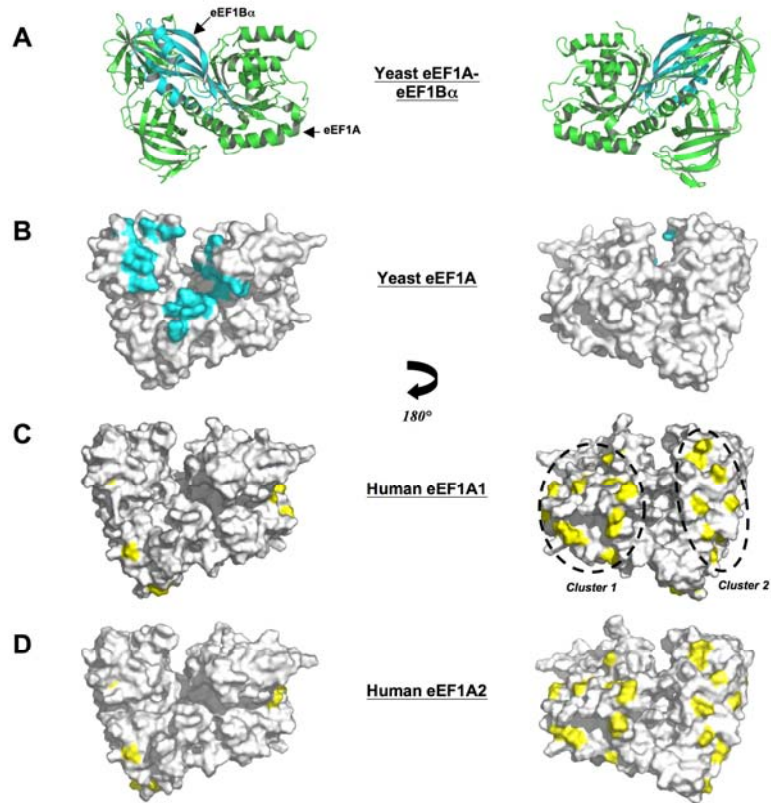
ZPR1 forms complexes preferentially with eEF1A-GDP, and in fact competes with the alpha subunit of eEF1B for binding (31). It is possible; therefore, that ZPR1 preferentially binds eEF1A2 as there is likely to be a consistently greater proportion of eEF1A2 in a GDP-bound form, compared to eEF1A1. It would have been an attractive hypothesis that ZPR1 was in fact the GTP exchange factor for eEF1A2; however, Mishra et al showed that ZPR1 does not modulate the intrinsic nucleotide binding kinetics of eEF1A (31). We are now studying aged mice which are heterozygous for the wasted mutation (*+/-wst*), to see if they also develop a late-onset form of motor neuron disease. It could be that eEF1A2 heterozygotes do not have sufficient eEF1A2 to interact with ZPR1 and thus to maintain motor neurons.

### Conclusions

The discovery that eEF1A exists as two distinct tissue-specific variants (32,33), and that the loss of eEF1A2 causes motor neuron degeneration has opened up new fields of study. Translation factors have generally been assumed to be ubiquitously expressed on the basis of their housekeeping functions, but it is increasingly clear that there are levels of complexity in the control of expression of these factors that remain to be discovered. The wasted mouse has proved to be a valuable tool in the study of eEF1A biology, and may also be a useful model for studying the processes involved in motor neuron degeneration.



**Figure 1: Changes in ventral (anterior) horn neurons of *wst/wst* mice.** The ventral horns of *wst/wst* (left) and wild-type (wt, right) are identified by squares in the upper panels. Higher magnifications of areas within the squares are shown in the lower panels. Motor neurons of the *wst/wst* mice are swollen with multiple cytoplasmic vacuoles.



**Figure 2: Location of eEF1B $\alpha$ -binding site on yeast eEF1A and variations in amino acids between human eEF1A1 and eEF1A2 mapped onto surface.** Two equivalent views rotated by 180° about the y-axis depicting: **(A)** a cartoon schematic representation of the yeast crystal structure (34) showing the C-terminal eEF1B $\alpha$  fragment (cyan) bound to eEF1A (green); **(B)** surface representations of the yeast eEF1A crystal structure with the location of the eEF1B $\alpha$ -binding site (cyan) mapped on it; **(C)** the three-dimensional model of human eEF1A1, and **(D)** human eEF1A2, with locations of surface-exposed variant amino acid side-chains mapped (yellow) [Soares et al., submitted]. The two sub-clusters are apparent in this representation, located on the opposite side from the known eEF1B $\alpha$ -binding site (34).

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